Samples with OD450 readings of less than 0.200 are considered negative in which case the analysis is complete, the results may be reported and the corresponding un-boiled SOLO+ media samples may be discarded following local regulations/guidelines.

Samples with OD450 ≥0.200 are considered presumptive positive for *Listeria*. Presumptive positive results must be verified using a recognised culture method.

9. CONFIRMATION OF POSITIVE RESULTS FROM *LISTERIA* IMMUNOASSAY

Samples with OD's ≥0.200 are considered positive for *Listeria*.

All samples identified as positive must be confirmed using the conventional tests described in the standardised methods by the FDA (BAM), USDA or ISO. The confirmation step must start from the (un-boiled) SOLO+ media samples stored at 30°C or 2-8°C.

Streak 10µl onto appropriate selective agar plates. Incubate agars as specified by standard *Listeria* cultural protocol. If typical *Listeria* colonies are observed on the plates this is sufficient to confirm the sample is positive for *Listeria* spp. Additional tests such as biochemical identification galleries can be used if there is any doubt about the validity of the result or to identify the species involved.

10. KIT STORAGE AND EXPIRY

The kit and any unused kit components should be stored at 2-8°C. DO NOT FREEZE.
The kit expiry date is displayed on the kit box plus all of the kit components within the box. Any unused diluted Washing buffer can be stored at 2-8°C for up to 10 days. Any unused microplate strips should be returned to the foil pouch with the desiccant sachet and the seal closed completely, then stored at 2-8°C.

11. SAFETY

The Stop Solution contains sulphuric acid which is corrosive. Wash immediately with large quantities of water if the solution comes into contact with skin or mucous membranes. For use in laboratory facilities with qualified and trained personnel for the handling of potentially pathogenic organisms.

Training is recommended to first time users and can be provided by Solus Scientific Solutions Ltd. As a guide, the following precautions should be taken as a minimum:

- Protective clothing should be worn including safety glasses, mask and gloves where appropriate.
- Avoid contact with skin.

12. PRECAUTIONS

- Reagents are provided at a matched working concentration. Optimum sensitivity and specificity will be reduced if reagents are modified or not stored under the recommended conditions.
- Do not mix different lots of reagents.
- Avoid microbial contamination of opened reagent bottles.
- Ensure that no cross contamination occurs between wells.
- Ensure that the Conjugate does not contaminate other reagents or equipment.
- Ensure that kit components are not exposed to temperatures greater than 40°C.
- Avoid using solutions containing sodium azide for cleaning of equipment especially washing devices (the peroxidase enzyme used in the kit is inactivated by sodium azide).
- Do not use for diagnostic purposes with medical specimens.

13. SDS INFORMATION

Safety data sheets (SDS) are available for this test on request.

14. WARRANTY

Accurate results depend on the proper use of the kit by following the instructions for use carefully. If the kit fails to perform according to specification please contact:

Tel +44 (0)1623 429701
Fax +44 (0)1623 620977
Email info@solusscientific.co

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UK

Issue number: **1**
Date: **05/18**
Product code: **LIS1-0096**

Food safety excellence

Solus **One** *Listeria*

Immunoassay Based Test System for the Detection of ***Listeria*** in Environmental Samples

www.solusscientific.com

Product Number: LIS1-0096
This method is for the next day detection of *Listeria* in environmental samples.

SOLUS SCIENTIFIC

1. INTRODUCTION

Solus One *Listeria* provides a negative or a presumptive positive result from a single enrichment step within 24 hours, of which the immunoassay is 2 hours and 45 minutes.

2. INTENDED USE

AOAC applicability is based on a validation study for sponge samplers with stainless steel environmental surfaces and swabs with plastic surfaces.
The test method requires laboratory facilities plus qualified and trained personnel. Training is recommended for first time users and can be provided by Solus Scientific Solutions Ltd. Using the method includes compliance with Good Laboratory Practices (refer to EN ISO 7218).

3. REAGENTS PROVIDED

Kit components are supplied stabilised and ready to use at working concentration. Only the Washing Buffer reagent requires dilution by addition of 10ml concentrated Washing Buffer reagent to 240ml of deionised water.

Each kit contains sufficient material for 1 x 93 determinations, plus controls. The kit expiry date is displayed on each product label.

- 1 x 96 well microplates (in breakable strip format). Wells are coated with antibodies against *Listeria* spp.
- Negative Control (Green label). 3ml in working dilution. Contains diluent with preservative.
- Positive Control (Red label). 3ml in working dilution. Contains heat-killed *Listeria* in diluent with preservative. The positive control is black in colour.
- Conjugate (Orange label). 11ml in working dilution. Contains horseradish peroxidase -antibody conjugate in diluent with preservative.
- Substrate (Blue label). 11ml in working dilution. Contains 3,3′,5,5′-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers. Solution is colourless or slightly faint blue.
- Stop Solution (Yellow label). 11ml in working dilution. Contains 10% sulphuric acid.
- Washing Buffer Concentrate (25x). 5 x 10ml.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Refrigerator at 2-8°C
- Deionised water
- SOLO+ medium pre-warmed to 30°C (Solus Scientific Cat # SOL001)
- Measuring cylinder for 250ml or 1L
- 1L vessel or Dynex wash container (if using Dynex DS2)
- Suitable enrichment containers
- Sterile 10ml test tubes/bottles
- 3ml transfer pipettes (sterile)
- Test tube for sample boiling (e.g 5ml Polypropylene rimless test tubes 13x75mm)
- Vortex mixer
- Timer
- Incubator or water bath at 30±1°C. Incubator at 37±1°C
- Heating block or water bath (capable of heating to 85-100°C)
- Pipettes and tips (1ml; 0.1ml)
- Dynex DS2
- OR Microplate reader with 450nm filter plus microplate washer or squeeze bottle
- Autoclave for decontamination of samples
- Sponge samplers or swabs soaked in suitable neutralising buffer (e.g. Letheen broth or Hi-cap buffer - World Bioproducts or equivalent)

5. REAGENT PREPARATION

Wash Buffer:

5.1 To a 250ml vessel add 10ml of concentrated Wash buffer reagent to 240ml of deionised water.

5.2 Transfer to a storage bottle and label the solution as appropriate.

Culture Medium:

5.3 Prepare the SOLO+ medium following manufacturer's instructions. Allow to cool to ambient temperature (e.g. 18 to 25 °C) before use in testing.

6. SAMPLE PREPARATION AND ENRICHMENT - standard method

SOLUS SCIENTIFIC

Selective enrichment:
Sample surface with sampling device. Follow sampling device manufacturers' instructions for correct use, storage and transport.

Warm SOLO+ to 30°C prior to use. Add 100ml SOLO+ to sponge sampler (e.g. World bio-products, Hygiena etc.) in a suitable bag. Add 10ml SOLO+ to a swab (e.g. sponge, foam or cotton). Hand massage or mix by vortexing and incubate for 22 -30 hours at 30±1°C. Ensure that bench processing time of inoculated SOLO+ media samples is kept to a minimum and transferred to 30°C incubation as soon as reasonably possible.

When the incubation period in SOLO+ is completed, carefully remove a 1ml aliquot, avoiding particulate matter to a glass or polypropylene test tube. Heat the aliquot to 85-100°C for 15-20 minutes in the test tube. After heating allow the sample to cool to room temperature. This may be accelerated by placing the test tubes in cold water for 5 minutes.

Retain the remaining un-boiled SOLO+ media samples for verification until immunoassay results are obtained. These samples can be kept at 30±1°C if the immunoassay test is to be carried out within 2 hours or at 2-8°C if not, It is possible to keep SOLO+ media samples for 72 hours at 2-8°C prior to the immunoassay test.

7. IMMUNOASSAY PROCEDURE

7.1. Take the test kit from storage at 2-8°C one hour before use to allow the components to reach room temperature. Determine the number of wells required for the test. Take the required number of strips from the pouch and fit them to the frame provided. Unused strips should be returned to the pouch and stored at 2-8°C.

7.2. Prepare Wash Buffer as detailed in section 5.1.

7.3. Leave the first well in the strip empty to serve as a 'blank' for measuring the absorbance of the substrate.

7.4. Pipette 0.1ml of Negative Control (Green label) into the second well.

7.5. Pipette 0.1ml of Positive Control (Red label) into the third well.

7.6. Pipette 0.1ml of each heat treated/cooled sample separately into consecutive wells in the strip. If there are wells left over at the end of a test strip the Positive or Negative Controls may be repeated.

7.7. Incubate the plate (containing the strips) at 37±1°C for 60 mins (±5 mins.) After incubation, aspirate the contents of the wells, removing as much of the liquid as possible. Wash the wells 5-7 times with Wash buffer, ensuring complete filling and emptying of the wells through each wash cycle. The washing technique is critical to assay performance, hence it is recommended to use a microplate washer.

7.8. Pipette 0.1ml of Conjugate (Orange label) into all wells except the 'blank'.

7.9. Incubate the plate at 37±1°C for 30 mins (±5 mins).

7.10. Repeat the wash cycles as detailed in section 7.7

7.11. Pipette 0.1ml of TMB Substrate (Blue label) into all wells, including the 'blank' well.

7.12. Incubate the plate at room temperature for 30 mins. (±5 mins).

7.13. After incubation, stop the reaction by adding 0.1ml of Stop Solution (Yellow label) to all wells including the 'blank' well. The Stop Solution will cause the blue colour in the wells to change to yellow.

7.14. Read the optical densities within 10 minutes in a plate reader using a 450nm filter. Inspect the wells before reading for air bubbles and if present burst with a needle. Zero the reader against the 'blank' well before the other wells are read.

7.15. Do not use reference filter.

8. INTERPRETATION OF RESULTS

Results are expressed as optical density (OD_{450}) measurements using microplate reader. Subtract the OD value of the blank well (usually A1) from all of the other results.

Assay acceptance criteria:

Criterion	Value
Negative Control OD_{450}	< 0.075
Positive Control OD_{450}	> 0.500

Should the value of Negative or Positive controls not meet these criteria, the test is not considered valid and must be performed again. Typical values for the negative control are 0.020 and for the positive control 0.900-1.700.